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Structural basis for major histocompatibility complex (MHC)-linked susceptibility to autoimmunity: Charged residues of a single MHC binding pocket confer selective presentation of self-peptides in pemphigus vulgaris

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ABSTRACT **Human T-cell-mediated autoimmune diseases are genetically linked to particular alleles of MHC class II genes. Susceptibility to pemphigus vulgaris (PV), an autoimmune disease of the skin, is linked to a rare subtype of HLA-DR4 (DRB1*0402, 1 of 22 known DR4 subtypes). The PV-linked DR4 subtype differs from a rheumatoid arthritis-associated DR4 subtype (DRB1*0404) only at three residues (DRβ 67, 70, and 71). The disease is caused by autoantibodies against desmoglein 3 (DG), and T cells are thought to trigger the autoantibody production against this keratinocyte adhesion molecule. Based on the DRB1*0402 binding motif, seven candidate peptides of the DG autoantigen were identified. T cells from four PV patients with active disease responded to one of these DG peptides (residues 190–204); two patients also responded to DG-(206–220). T-cell clones specific for DG-(190–204) secreted high levels of interleukins 4 and 10, indicating that they may be important in triggering the production of DG-specific autoantibodies. The DG-(190–204) peptide was presented by the disease-linked DRB1*0402 molecule but not by other DR4 subtypes. Site-directed mutagenesis of DRB1*0402 demonstrated that selective presentation of DG-(190–204), which carries a positive charge at the P4 position, was due to the negatively charged residues of the P4 pocket (DRβ 70 and 71). DRβ 71 has a negative charge in DRB1*0402 but a positive charge in other DR4 subtypes, including the DR4 subtypes linked to rheumatoid arthritis. The charge of the P4 pocket in the DR4 peptide binding site therefore appears to be a critical determinant of MHC-linked susceptibility to PV and rheumatoid arthritis.**

The MHC is an important susceptibility locus for human autoimmune diseases, such as insulin-dependent diabetes, rheumatoid arthritis (RA), and pemphigus vulgaris (PV) (reviewed in refs. 1–4). A genome-wide search for type I diabetes susceptibility genes with microsatellite markers confirmed that the MHC is the most important susceptibility locus and that a number of other genes contribute to the disease process (5). Disease-linked polymorphisms map to the peptide binding site of MHC molecules (6, 7), indicating that peptide presentation to T cells may be important in the initiation or progression of these diseases. The HLA-DR4-linked autoimmune diseases, RA and PV, may offer an opportunity to define the structural basis of MHC-linked disease susceptibility. Susceptibility to autoimmunity in RA and PV is linked to MHC class II molecules that differ only in a small, defined region of the peptide binding site (3, 4, 8–10).

PV is a life-threatening blistering disease of the skin and mucous membranes (11). Autoantibodies specific for a keratinocyte cell adhesion molecule, desmoglein 3 (DG), cause a loss of cell adhesion and blister formation. DG is a member of the cadherin family of Ca^{2+}-dependent cell adhesion molecules (12). The autoantibodies are pathogenic as neonates of mothers with PV have a transient blistering skin disease due to maternal IgG that crosses the placenta (13). A loss of cell adhesion and blister formation are observed when IgG or affinity-purified DG-specific antibodies from PV patients are transferred to neonatal mice by i.p. injection (14–16).

Susceptibility to PV is linked to the DRB1*0402 haplotype, 1 of 22 known DR4 haplotypes. Among Ashkenazi Jews, >90% of PV patients carry the DRB1*0402 haplotype, which is rare in the general population. The DQ8 genes (DQA1*0301, DQB1*0302) of this haplotype are also found in haplotypes not associated with susceptibility to PV (i.e., DRB1*0401), indicating that the primary association is with the DRB1*0402 gene (9, 17, 18).

In other ethnic groups (non-Ashkenazi Jews, Caucasians, Japanese) susceptibility is linked to a rare DQ1 allele (DQB1*0503) (17, 19, 20). The DQB1*0503 allele is structurally very interesting since it differs from a common DQB1 allele (DQB1*0501) only by a valine to aspartic acid substitution of DQβ 57. The DQβ 57 position is also important in the diabetes-linked DQ alleles (DQB1*0302, DQB1*0201). In contrast to the PV-linked DQ allele, where Asp-57 *confers* susceptibility, susceptibility to diabetes is linked to alleles that do *not* carry a negative charge at DQβ 57 (2, 21, 22). Polymorphisms of particular MHC class II pockets (P4 pocket in DR4 molecules, P9 pocket in DQ molecules) therefore appear to be important in determining susceptibility to several different human autoimmune diseases.

MATERIALS AND METHODS

Methods for generating human T-cell clones and for examining their MHC/peptide specificity were as described (23).

RESULTS

Structural Motif for Peptides Presented by the PV-Associated DR4 Molecule. The PV-associated DR4 subtype (DRB1*0402) carries a negative charge at DRβ 70 and 71; in the DR1 crystal structure these residues are located in the P4 pocket of the peptide binding site. Most DR4 subtypes (except DRB1*0402 and 0414) have a positive charge (lysine or arginine) at DRβ 71. Residues that are polymorphic between the DR4 subtypes are listed for DRB1*0401 to 0412 in Table 1; the other known DR4 subtypes (DRB1*0413 to 0422) are

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Abbreviations: MHC, major histocompatibility complex; PV, pemphigus vulgaris; IL, interleukin; RA, rheumatoid arthritis; DG, desmoglein 3; r, recombinant; EBV, Epstein–Barr virus.

Table 1. Polymorphic residues of HLA-DR4 subtypes

	DRβ	0401	**0402**	0403	0404	0405	0406	0407	0408	0409	0410	0411	0412
	37	Tyr	Tyr	Tyr	Tyr	Tyr	Ser	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr
P9 pocket	57	Asp	Asp	Asp	Asp	Ser	Asp	Asp	Asp	Ser	Ser	Ser	Ser
	67	Leu	Ile	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Ile
	70	Gln	**Asp**	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Asp
P4 pocket	71	Lys	**Glu**	Arg	Arg	Arg	Arg	Arg	Arg	Lys	Arg	Arg	Arg
	74	Ala	Ala	Glu	Ala	Ala	Glu	Glu	Ala	Ala	Ala	Glu	Leu
P1 pocket	86	Gly	Val	Val	Val	Gly	Val	Gly	Gly	Gly	Val	Val	Val

The PV-associated DR4 molecule (DRB1*0402) has a negative charge at DRβ 70 and 71; other DR4 subtypes have a positive charge at DRβ 71 (lysine or arginine). The DR4 molecules associated with RA (DRB1*0401 and 0404) therefore have a positively charged P4 pocket, while the PV-associated DR4 molecule (DRB1*0402) has a negatively charged P4 pocket.

very rare (24). DRB1*0402 and 0414 differ only by the Val/Gly dimorphism that controls the size of the P1 pocket.

The comparison of DRB1*0402 (PV linked) and 0404 (RA linked) is particularly informative as these alleles differ only at three positions (DRβ 67, 70, and 71). DRB1*0404 confers an increased risk for RA, indicating that the DRβ 67–71 segment is critical for determining susceptibility to these two different autoimmune diseases (8, 18).

The crystal structure of HLA-DR1 demonstrated that DRβ 70 and 71 contribute to the shape and charge of a pocket that accommodates the P4 side chain of the bound peptide (the P1 side chain is the first DR anchor residue) (7) (Fig. 1). This suggested that DRβ 70 and 71 may confer an increased risk to RA and PV by allowing the binding of self-peptides with a particular shape/charge at P4. Peptide binding studies demonstrated that peptides with a charge complementary to that of DRβ 71 were selective for either the RA- or the PV-associated DR4 molecules (10). Based on these considerations and on the previously established preferences for the P1 and P6 pockets (25), a set of criteria was developed for the identification of candidate peptides from the known target antigen, DG (4). The DRB1*0402 binding criteria considered the preference for hydrophobic residues (V, L, I, M, F) at P1, small side chains at P6 (S, T, N, V), and positively charged residues at P4 (K, R). Seven peptides of DG matched this structural motif (Table 2). Six of these peptides were located in the extracellular domain that is recognized by DG-specific autoantibodies (12).

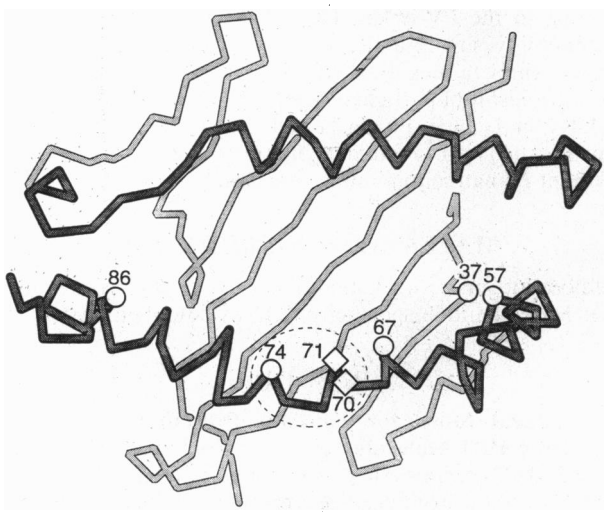

FIG. 1. Position of polymorphic DRβ chain residues in the HLA-DR peptide binding site. Residues DRβ 70 and 71 have a negative charge in the pemphigus-associated DRB1*0402 molecule and are located in the P4 pocket (circled) (7). DRB1*0402 and the closely related 0414 are the only DR4 alleles that have negatively charged residues at DRβ 70 and 71 of the P4 pocket. All other DR4 subtypes have a positive charge at DRβ 71. The negative charge of DRβ 70 and 71 is responsible for the selective presentation of self-peptides with a positive charge at P4.

Recognition of DG Peptides by T Cells from PV Patients. In PV, the clinical course is marked by relapses and remissions; disease activity correlates with the production of DG-specific autoantibodies (12, 15) against an immunodominant epitope of the extracellular domain, residues 200–229 (16). T-cell responses to the seven DG peptides were evaluated in four patients with active disease. Patients had clinical features of PV; the diagnosis was confirmed by histology and immunohistology of the skin. T-cell lines were raised against the DG peptides from blood mononuclear cells using peptides at a concentration of 5 μM. T-cell lines were expanded by addition of recombinant interleukin 2 (rIL-2) and tested for their specificity after 12–14 days of culture; T-cell lines with a stimulation index of >3 [T-cell proliferation in the presence of peptide (cpm)/T-cell proliferation in the absence of peptide (cpm)] were considered positive. From each patient, DG-(190–204)-specific T-cell lines were obtained that showed a strong proliferative response in the initial assay (stimulation index >10). Two patients also showed a response to the DG-(206–220) peptide and one patient responded to DG-(251–265) or DG-(762–786). No T-cell responses were seen to peptides DG-(78–93), DG-(97–111), and DG-(512–526) (Table 3). These data demonstrate that at least one DG peptide (residues 190–204) is a target for autoreactive T cells in PV patients with active disease. The DG-(190–204) and DG-(206–220) T-cell epitopes are adjacent to each other in the extracellular domain of DG and overlap with an immunodominant antibody epitope of the extracellular domain, DG-(200–229) (16).

It is not yet known whether the DG-(190–204) peptide is the immunodominant T-cell epitope of DG. This question may be

Table 2. The binding motif of the PV-associated DR4 molecule (encoded by DRA, DRB1*0402 genes) was used to define peptides of the DG autoantigen

Motif	1	4	6
	V	**K**	**S**
	L	**R**	**T**
	I		**N**
	M		**V**
	F		
DG-(78–93)	ATQK**I**TY**R**I**S**GVGID		
DG-(97–111)	FGIF**V**VD**K**N**T**GDINI		
DG-(190–204)	LNSK**I**AF**K**I**V**SQEPA		
DG-(206–220)	TPMF**L**LS**R**N**T**GEVRT		
DG-(251–265)	CECN**I**KV**K**D**V**NDNFP		
DG-(512–526)	SART**L**NN**R**Y**T**GPYTF		
DG-(762–786)	QSGT**M**RT**R**H**S**TGGTN		

In this motif, the MHC anchor positions P1, P4, and P6 were considered. Peptide residue P4 carries a positive charge (K or R) due to the negative charge of the P4 pocket in the PV-associated DR4 molecule.

Table 3. T-cell responses to DG peptides in PV patients

Peptide	Patient Go		Patient Ro		Patient DC		Patient St	
	No peptide	Plus peptide	No peptide	Plus peptide	No peptide	Plus peptide	No peptide	Plus peptide
DG-(78–93)	—	—	—	—	—	—	—	—
DG-(97–111)	—	—	—	—	—	—	—	—
DG-(190–204)	99	18,066	558	10,727	10,517*	112,146	81	3554
	223	3,498	301	3,172				
	82	1,781	154	1,410				
	231	1,515	307	1,963				
	254	2,104	425	2,597				
	276	941	4833	25,934				
DG-(206–220)	—	—	106	4,848	10,674	62,438	—	—
			2705	11,210	12,296	57,851		
			2138	7,634	20,590	66,995		
DG-(251–265)	—	—	116	3,943	—	—	—	—
			188	1,641				
DG-(512–526)	—	—	—	—	—	—	—	—
DG-(762–786)	121	5,782	—	—	—	—	—	—
	31	1,128						
	693	2,443						

T-cell lines specific for DG peptides were established from four PV patients with active disease. Blood mononuclear cells were plated at 10^5 cells per well of a U-bottom microtiter plate; typically 24 wells were set up for each individual peptide (5 μM peptide). rIL-2 was added on day 3 and the specificity of T-cell lines was examined in a proliferation assay on days 12–14. T-cell lines with a stimulation >3 were considered positive. T-cell lines with a strong proliferative response to DG-(190–204) (stimulation index >10) were obtained from each patient. No T-cell responses were seen with peptides DG-(78–93), DG-(97–111), and DG-(512–526). Ten wells were set up for each peptide with cells from patient DC, 24 wells per peptide for the other PV cases. T-cell lines specific for DG-(190–204) were set up twice for patient Go (24 wells in the first experiment, 96 in the second) and a total of six DG-(190–204)-specific lines was obtained. The MHC haplotypes of the patients were as follows: patient Go, DRB1*0402, DQB1*0302; patient DC, DRB1*0402, DRB1*0403, DQB1*0302; patient St, DRB1*0402, DRB1*1401, DQB1*0302, DQB1*05031; patient Ro, DRB1*03011, DRB1*1401, DQB1*0201, DQB1*05031.

*The background was high with this patient as autologous Epstein–Barr virus (EBV)-transformed B cells were used as antigen presenting cells for this experiment, while autologous nontransformed mononuclear cells were used in the other cases.

addressed by generating T-cell clones specific for recombinant DG and by determining the epitope specificity of these clones. Since the DRB1*0402 haplotype is uncommon in the general population (≈1% of the general population in the United States), T-cell reactivity to DG and DG peptides can be compared between patients and healthy family members who share the DRB1*0402 haplotype.

Selective Presentation of a DG Peptide by the PV-Linked DR4 Molecule (DRB1*0402). DG-(190–204) and DG-(206–220) specific T-cell lines were HLA-DR restricted as T-cell proliferation was blocked by a monoclonal antibody (mAb) specific for HLA-DR (L243) but not by a mAb specific for HLA-DQ (G2a.5, specific for DQ1, DQ7, and DQ8) (data not shown). T-cell lines specific for DG-(190–204) were cloned by limiting dilution to determine if presentation of this peptide was selective for the PV-linked DRB1*0402 molecule. The DG-(190–204) peptide was presented only by a B-cell line homozygous for DRB1*0402 but not by B cells homozygous for the RA-associated haplotypes (DRB1*0401 and 0404) or other DR4 haplotypes (DRB1*0403, 0405, 0406, 0407, 0408) (Fig. 2). Selective presentation of DG-(190–204) by DRB1*0402 was confirmed using L cell transfectants that expressed DRB1*0401, 0402 or 0404 (Fig. 3). The fact that the peptide was presented by DRB1*0402 but not by DRB1*0404 was particularly informative as these molecules differ only at three polymorphic positions, DRβ 67, 70, and 71.

Residues DRβ 70 and 71 of the P4 Pocket Are Responsible for Selective Presentation of a DG Peptide. Residues critical for peptide presentation were determined by site-directed mutagenesis of DRβ 67, 70, and 71 in the DRB1*0402 cDNA (26). T-cell recognition of the peptide was abolished when glutamic acid at DRβ 71 was replaced by a positively charged amino acid, lysine (as in 0401 or 0409) or arginine (other DR4 subtypes) (Fig. 4). The peptide was also not presented when aspartic acid at DRβ 70 was replaced by glutamine (present in the majority of DR4 subtypes). Substitution of isoleucine at DRβ 67 by leucine only resulted in a moderate reduction of the T-cell response. Thus, two negatively charged residues of the P4 pocket (DRβ 70 and 71) are critical for presentation of DG-(190–204), providing a structural basis for DRB1*0402-linked autoimmunity to PV.

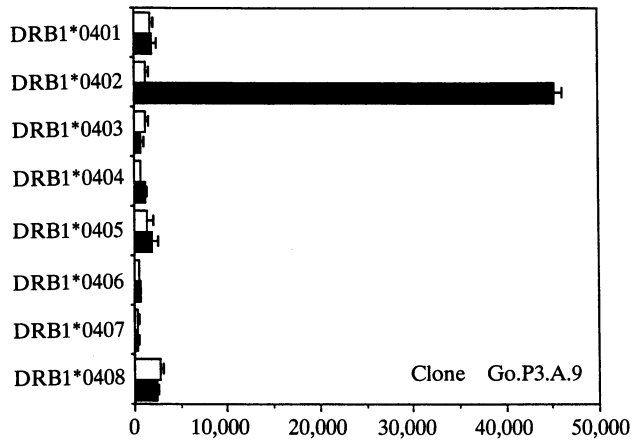

FIG. 2. Presentation of DG-(190–204) is selective for the PV-linked DR4 molecule (DRB1*0402). EBV-transformed B-cell lines [Priess (DRB1*0401), YAR 634 (DRB1*0402), SSTO (DRB1*0403), PE 636 (DRB1*0404), JH22798 (DRB1*0405, DRB1*1301), IUU 020 (DRB1*0406), JHAF (DRB1*0407), and MT 706 (DRB1*0408, DRB1*0404)] were used as antigen-presenting cells for a DG-(190–204)-specific T-cell clone. B cells were pulsed for 18 hr with 20 μM DG-(190–204), washed, irradiated, and cocultured for 3 days with DG-(190–204)-specific T-cell clones (5×10^4 T cells and B cells per well, in triplicates). □, No peptide; ■, plus DG-(190–204). T-cell proliferation was determined by ^{3}[H]thymidine incorporation. Presentation of the DG-(190–204) peptide was specific for the PV-linked DRB1*0402 molecule. DRB1*0402 differs only at three positions from the DRB1*0404 molecule associated with susceptibility to RA, indicating that residues DRβ 67, 70, and 71 are important in defining susceptibility to these two different autoimmune diseases.

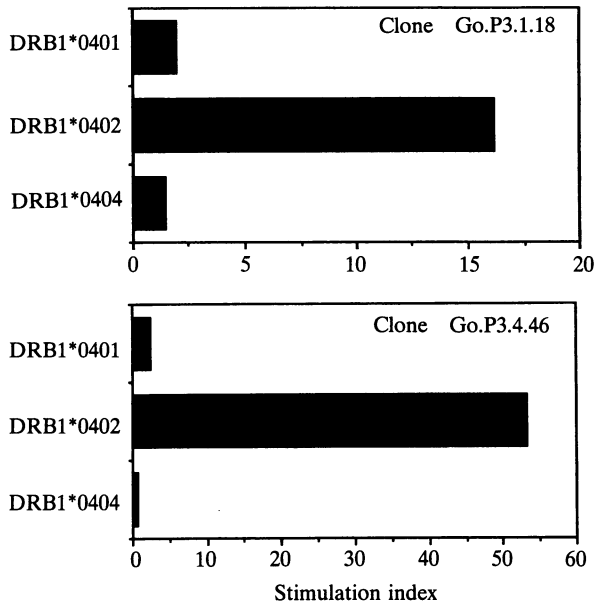

FIG. 3. Presentation of DG-(190–204) by DRA, DRB1*0402 transfectants. L cells expressing the PV-associated DRB1*0402 or the RA-associated DRB1*0401 or 0404 molecules were used as antigen-presenting cells for two DG-(190–204)-specific T-cell clones from a PV patient. L-cell transfectants were pulsed with the DG-(190–204) peptide at 10 μM, washed, irradiated, and cocultured with DG-(190–204)-specific T-cell clones for 72 hr (10^5 L cells, 5×10^4 T cells per well, in triplicates). T-cell proliferation was quantitated by ^{3}[H]thymidine incorporation. Numbers represent the stimulation index (cpm in the presence of peptide/cpm in the absence of peptide).

T-Cell Clones Specific for DG (Residues 190–204) Secrete IL-4 and IL-10. DG-specific autoantibodies interfere with keratinocyte cell adhesion and thereby induce the severe skin blistering seen in PV patients (12). Induction of autoantibody production by DG-specific T cells would require the production of cytokines that induce the activation and differentiation of autoreactive B cells. IL-4 and IL-10 are important cytokines that are secreted by Th2 cells and that promote B-cell differentiation and antibody production (reviewed in ref. 27).

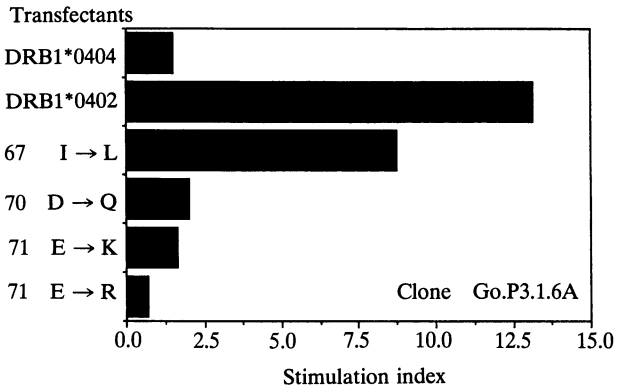

FIG. 4. Residues 70 and 71 of the DRβ chain are responsible for the selective presentation of DG-(190–204) by DRB1*0402. The DG-(190–204) peptide was presented by the PV-associated DRB1*0402 molecule but not by the RA-associated DRB1*0404 molecule, which differ only at DRβ 67, 70, and 71. DRβ 67, 70, and 71 of DRB1*0402 were mutated to those residues found in DRB1*0404. L-cell transfectants that expressed these molecules were used as antigen-presenting cells in a T-cell proliferation assay. Numbers represent the stimulation index (cpm in the presence of peptide/cpm in the absence of peptide). The data demonstrate that DRβ 70 and 71 of the P4 pocket confer selective presentation of the DG-(190–204) peptide.

To examine antigen-driven cytokine production of the DG-(190–204)-specific T-cell clones, T cells were cocultured with autologous blood mononuclear cells as antigen-presenting cells. Cytokine content of supernatants was monitored after 48 hr by ELISA. Two of three clones tested secreted high levels of IL-4; one of the clones also secreted large amounts of IL-10 (Table 4). No detectable quantities of IL-4 or IL-10 were secreted in the absence of antigen. Antigen-driven IL-4 and IL-10 secretion by these T-cell clones may be important in the activation and differentiation of B cells that produce DG-specific autoantibodies.

DISCUSSION

Polymorphic residues that shape the P4 pocket of HLA-DR4 molecules appear to be critical in determining susceptibility to two different autoimmune diseases, RA and PV. Why is the P4 pocket so important? The P4 pocket is located in a central position of the HLA-DR peptide binding site and is flanked by residues that act as primary T-cell receptor contact residues (P2, P3, and P5, as demonstrated for an immunodominant myelin basic protein peptide) (7, 23, 28, 29). The P4 pocket has a surface that appears to be extremely variable (only 23% conserved) while the surface of the P6 pocket is more conserved (49%) (33). In contrast, the P1 pocket is shaped mostly by residues of the nonpolymorphic DRα chain and is occupied by hydrophobic anchor residues in all DR molecules (6, 7). Among the polymorphic residues of the P4 pocket, the charge at DRβ 71 is critical for the selective binding of self-peptides to the RA- and PV-linked DR4 molecules (4, 10). The negative charge of DRβ 70 and 71 in the PV-linked DRB1*0402 molecule confers selective binding to self-peptides that have a positive charge at P4 [a lysine in DG-(190–204) and an arginine in DG-(206–220)]. The positive charge of DRβ 71 in the RA-associated DR4 molecules confers selective binding of peptides with a negative charge at P4.

The self-peptides recognized by autoaggressive T cells in human autoimmune diseases have been difficult to identify. For many of the common autoimmune diseases (RA, insulin-dependent diabetes, multiple sclerosis) the target antigens are not known, although candidate antigens have been identified (i.e., type II collagen in RA, glutamic acid decarboxylase 65 in type I diabetes, and myelin basic protein and proteolipid protein in multiple sclerosis). The identity of target proteins has been established for those autoimmune diseases in which autoantibodies interfere with specific cellular functions (i.e., neuromuscular transmission in myasthenia gravis, control of thyroid hormone secretion in Grave disease) (30). T-cell

Table 4. Antigen-driven IL-4 and IL-10 secretion was examined for three DG-(190–204)-specific T-cell clones from a PV patient

Clone	No antigen	20 μM	10 μM	5 μM
	IL-4 secretion, pg/ml			
Go.P3.1.6A	<10	980	330	145
Go.P3.4.22	<10	190	103	<10
Go.P3.A.9	<10	420	330	560
	IL-10 secretion, pg/ml			
Go.P3.1.6A	<10	1450	630	450
Go.P3.4.22	<10	180	95	77
Go.P3.A.9	<10	78	148	230

T cells (5×10^4 per well) were cocultured with autologous mononuclear cells (10^5 per well) for 48 hr; synthetic peptide was added to 5, 10, or 20 μM. Cytokine content of supernatants was determined by sandwich ELISA (PharMingen) using rIL-4 and rIL-10 as standards. Two T-cell clones secreted large amounts of IL-4; one of the clones also secreted large quantities of IL-10. Cytokine secretion was not detectable in the absence of antigen. Antigen-driven IL-4 and IL-10 secretion by DG-specific T cells may be important in inducing the production of DG-specific autoantibodies in PV.

recognition of some of these autoantigens has been studied; however, it is not clear which of the T-cell epitopes identified are important in the disease process as no clear structural relationship between the disease-associated MHC molecules and self-peptides from these autoantigens has emerged. In PV, the situation is unique because (*i*) the autoantigen has been identified and the immunological effector mechanisms have been established (11–15), (*ii*) the linkage of disease susceptibility to the DRB1*0402 or the DQB1*0503 haplotype is very strong (9, 17–19), and (*iii*) unique structural features of the DRB1*0402 peptide binding site can be defined since there are many structurally related DR4 subtypes that are not associated with the disease (Table 1). The fact that the disease-linked polymorphisms shape the specificity of a pocket of the DRB1*0402 peptide binding site strongly suggests that peptide presentation to T cells is important in the pathogenesis of PV. The observation that a DG peptide with a positive charge at P4 is presented by DRB1*0402 but not by other DR4 subtypes may explain why the disease is associated with this particular MHC class II molecule.

A striking aspect of the two DR4-linked autoimmune diseases is that RA and PV are clinically and immunologically so different, even though the disease-associated DR4 molecules differ in a small, albeit strategic, location. In RA the joint destruction is thought to result from a chronic Th1-mediated autoimmune reaction against the synovial lining of the joint (31); in PV the disease is probably caused by Th2 cells that induce the production of DG-specific autoantibodies (11). In the skin, keratinocytes can act as antigen-presenting cells since they express MHC class II molecules following exposure to γ-interferon. Keratinocytes that express MHC class II molecules (i.e., following local inflammation or UV exposure) may therefore present DG peptides to T cells; Langerhans cells of the skin may also be involved in the presentation of the autoantigen. Keratinocytes are known to skew T-cell responses to a Th2 cytokine profile (IL-4 and IL-10) due to a failure to produce sufficient quantities of IL-12 (reviewed in ref. 32). The local mechanisms of antigen presentation in the skin and in the joint may therefore be critical in inducing Th2- or Th1-mediated autoimmune responses that produce such different clinical manifestations in PV and RA.

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